Programmed cell death[*]

Barbara Conradt[§], Department of Genetics, Dartmouth Medical School, Hanover, NH 03755 USA

Ding Xue[†], Department of MCD Biology, University of Colorado, Boulder, CO 80309 USA

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Abstract

    Programmed cell death is an integral component of *C. elegans* development. Genetic studies in *C. elegans* have led to the identification of more than two dozen genes that are important for the specification of which cells should live or die, the activation of the suicide program, and the dismantling and removal of dying cells. Molecular and biochemical studies have revealed the underlying conserved mechanisms that control these three phases of programmed cell death. In particular, an interplay of transcriptional regulatory cascades and networks involving CES-1, CES-2, HLH-1/HLH-2, TRA-1, and other transcriptional regulators is crucial in activating the expression of the key death-inducing gene *egl-1* in cells destined to die. A protein interaction cascade involving EGL-1, CED-9, CED-4 and CED-3 results in the activation of the key cell death protease CED-3. The activation of CED-3 initiates the cell disassembly process and nuclear DNA fragmentation, which is mediated by the release of apoptogenic mitochondrial factors (CPS-6 and WAH-1) and which involves multiple endo- and exo-nucleases such as NUC-1 and seven CRN nucleases. The recognition and removal of the dying cell is mediated by two partially redundant signaling pathways involving CED-1, CED-6 and CED-7 in one pathway and CED-2, CED-5, CED-10, CED-12 and PSR-1 in the other pathway. Further studies of programmed cell death in *C. elegans* will continue to advance our understanding of how programmed cell death is regulated, activated, and executed in multicellular organisms

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1. Introduction

Genetic studies of programmed cell death, or apoptosis, in *C. elegans* led to the identification of key players involved in this important physiological process from *C. elegans* to humans (Adams, 2003; Danial and Korsmeyer, 2004; Horvitz, 2003). These pioneering studies were made possible by the biology of *C. elegans*: 1. Unlike in many other animals, programmed cell death is not essential for *C. elegans* viability, at least under laboratory conditions (Ellis and Horvitz, 1986); 2. cells undergoing programmed cell death in *C. elegans* change their morphology and refractivity and can be observed in living animals using Differential Interference Contrast microscopy (DIC), also referred to as Nomarski optics; (Figure 1; Robertson and Thomson, 1982); 3. programmed cell death that occurs during *C. elegans* development is determined by the essentially invariant somatic cell lineage of *C. elegans*; therefore, it is not only known which cells undergo programmed cell death but also when and where they die (Sulston and Horvitz, 1977; Sulston et al., 1983). These unique features made it possible to genetically dissect the process of programmed cell death in *C. elegans* at single cell resolution. The resulting ground-breaking work was recognized with the Nobel Prize for Medicine in 2002, which was awarded to Sydney Brenner, John E. Sulston, and H. Robert Horvitz for their leading roles in deciphering the *C. elegans* cell lineage and in defining the genetic pathway of programmed cell death (Brenner, 2003; Horvitz, 2003; Sulston, 2003).
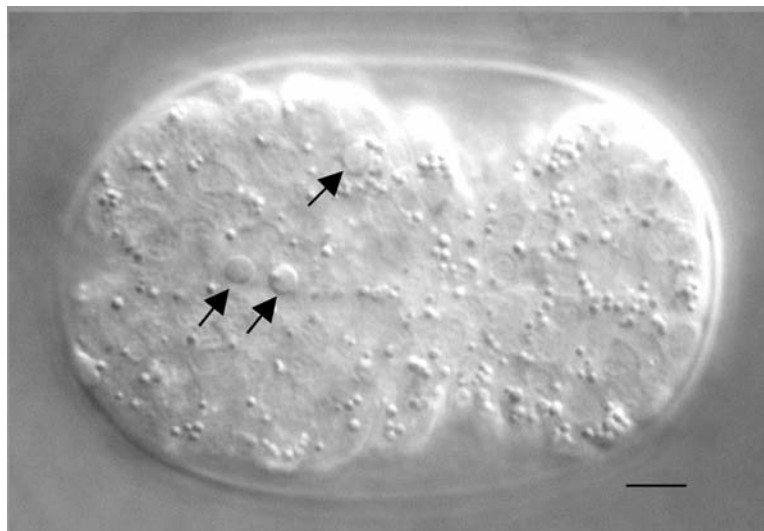

Figure 1. Nomarski image of an embryo with apoptotic cells. Three cells indicated by arrows underwent programmed cell death in a bean/comma stage embryo and exhibit a refractile, raised-button-like appearance. The bar represents 5 μm.

Programmed cell death occurs during two stages of *C. elegans* life and in two different types of tissues: during embryonic and post-embryonic development of the soma (referred to as "developmental cell death"; Sulston and Horvitz, 1977; Sulston et al., 1983) and in the gonad of adult hermaphrodites (referred to as "germ cell death"; Gumienny et al., 1999; Sulston, 1988; White, 1988). Programmed cell death proceeds in three genetically distinguishable phases: during the "specification phase", a cell is instructed to undergo programmed cell death; in the "killing phase", the apoptotic program is activated in the cell instructed to die; during the "execution phase", cells are dismantled and subsequently engulfed by neighboring cells (Horvitz, 1999; Figure 2). Mutations that lead to a partial block in this final phase, such as mutations in the genes *ced-1* and *ced-2* (Hedgecock et al., 1983), result in the accumulation of dead cells (referred to as "cell corpses"). Mutations in *ced-1* and *ced-2* were the first mutations to be identified as affecting programmed cell death and they were instrumental in the subsequent identification of genes involved in apical phases of programmed cell death. In the following, we will review our current understanding of the genes involved in the specification, killing, and execution phases in the case of developmental cell death. Germ cell death is discussed elsewhere in this book (see Germline survival and cell death).

2. Killing phase

2.1. The core machinery involved in the activation of the apoptotic program

Three death-promoting genes, *egl-1* (*egl*, egg-laying defective), *ced-3* (*ced*, cell-death abnormal) and *ced-4*, are required for most, if no all, developmental cell death in *C. elegans*. Strong loss-of-function (lf) mutations in any of these genes result in the survival of essentially all cells that normally undergo programmed cell death during development (Conradt and Horvitz, 1998; Ellis and Horvitz, 1986). Furthermore, these three genes act within dying cells to promote apoptosis, indicating that cells die by an intrinsic suicide mechanism (Conradt and Horvitz, 1998; Shaham and Horvitz, 1996a; Yuan and Horvitz, 1990). In contrast, the activity of the *ced-9* gene protects cells from undergoing programmed cell death during *C. elegans* development (Hengartner et al., 1992). Loss-of-function mutations in *ced-9* cause embryonic lethality, as a consequence of the ectopic deaths of cells that normally live. *ced-3*, *ced-4*, *egl-1*, and *ced-9* appear to act in a simple genetic pathway in which *egl-1* acts upstream of *ced-9* to induce cell death, *ced-9* acts upstream of *ced-4* to inhibit cell death, and *ced-4* acts upstream of *ced-3* to kill cells (Conradt and Horvitz, 1998; Hengartner et al., 1992; Shaham and Horvitz, 1996a; Figure 2).
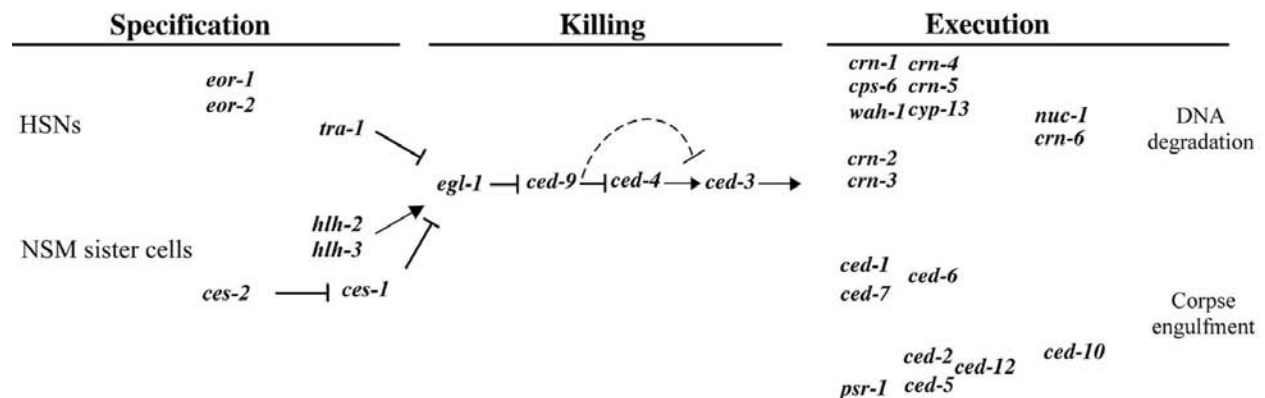
Figure 2. Genetic pathway of programmed cell death in *C. elegans*. Three phases of programmed cell death, **specification**, **killing**, and **execution**, are indicated. In the "**specification**" phase, genes involved in regulating the death fates of specific cells (HSNs and NSM sister cells) are shown. In the "**execution**" phase, two partially redundant pathways mediate the engulfment of cell corpses and the fragmentation of chromosomal DNA. *nuc-1* and *crn-6* may be involved in the degradation of DNA debris from apoptotic cells.

ced-9 encodes a protein similar to the product of the human proto-oncogene *bcl-2* (Hengartner and Horvitz, 1994), which plays a similar role in preventing apoptosis in mammals (Adams and Cory, 2001). *ced-9* and *bcl-2* are members of a gene family that plays important roles in regulating apoptosis in diverse organisms (Adams and Cory, 2001; Reed, 1997). *egl-1* encodes a small protein of 91 amino acids with a BH3 (BH3, Bcl-2 homology region 3) motif, which has been found in all pro-apoptotic members of the Bcl-2 gene family and mediates direct binding of these proteins to anti-apoptotic Bcl-2 members (Bouillet and Strasser, 2002; Conradt and Horvitz, 1998). *ced-3* encodes the founding member of a family of aspartate-specific cysteine proteases named caspases (Alnemri et al., 1996; Yuan et al., 1993). Like other caspases, CED-3 is synthesized as a proenzyme and is proteolytically activated to generate an active protease containing a p13 and p17 subunit (Alnemri et al., 1996; Xue et al., 1996). CED-3 protease activity appears to be essential for *ced-3* to cause programmed cell death in *C. elegans* (Xue et al., 1996). *ced-4* encodes a protein similar to human Apaf-1 (apoptotic protease activating factors), an activator of human caspase-9 (Yuan and Horvitz, 1992; Zou et al., 1997). Both CED-4 and Apaf-1 contain a caspase-recruitment domain (CARD domain) and nucleotide-binding motifs that are critical for the function of these proteins (Seshagiri and Miller, 1997; Zou et al., 1999). By analogy, CED-4 may play a role in activating CED-3 during apoptosis. Interestingly, *ced-4* may also produce an alternatively spliced transcript, *ced-4L*, which encodes a slightly larger protein (CED-4L) with a twenty-four amino acid insertion between its two nucleotide-binding motifs and which might protect against programmed cell death (Shaham and Horvitz, 1996b).

2.2. Biochemical model for the activation of the apoptotic program

Biochemical and cell biological analyses of EGL-1, CED-9, CED-4, and CED-3 have provided important insights into how these proteins function to regulate the activation of programmed cell death during *C. elegans*

development (Horvitz, 2003). CED-4 has been shown to physically interact with CED-9 *in vitro* and in cultured cells (Chinnaiyan et al., 1997; Spector et al., 1997; Wu et al., 1997). Furthermore, endogenous CED-9 and CED-4 proteins co-localize at the surface of mitochondria in *C. elegans* embryos and the mitochondrial localization of CED-4 is dependent on CED-9 (Chen et al., 2000). In addition to CED-9, CED-4 has been shown to interact with CED-3 *in vitro* and in mammalian cells and it has been proposed that a ternary CED-9/CED-4/CED-3 complex might form *in vivo* (Chinnaiyan et al., 1997; Yang et al., 1998). However, the subcellular localization of endogenous CED-3 has not been determined. Ectopic *egl-1* expression in *C. elegans* embryos results in the translocation of CED-4 to perinuclear membranes and ectopic programmed cell death (Chen et al., 2000). CED-4 translocation from mitochondria to perinuclear membranes appears to be initiated by the binding of EGL-1 to CED-9, which induces a major conformational change in the CED-9 protein (Yan et al., 2004), resulting in the release of CED-4 from the CED-9/CED-4 complex (Conradt and Horvitz, 1998; del Peso et al., 1998; Parrish et al., 2000). A gain-of-function mutation in *ced-9 (n1950)*, which results in the substitution of glycine 169 with glutamate and which blocks most, if not all programmed cell death during development, impairs the binding of EGL-1 to CED-9 and EGL-1-induced release of CED-4 (Parrish et al., 2000; Yan et al., 2004). EGL-1-induced CED-4 translocation is thought to be important for the activation of CED-3 and hence programmed cell death (Chen et al., 2000). CED-4 translocation may promote CED-4 self-oligomerization, which is thought to bring CED-3 proenzymes to close proximity for self-activation (Yang et al., 1998). The only *C. elegans* substrate of CED-3 known so far is CED-9 (Xue and Horvitz, 1997). CED-9 may not only inhibit cell death activation by sequestering CED-4 to mitochondria but also by acting as a competitive inhibitor of CED-3 (Xue and Horvitz, 1997). The mechanism by which CED-3 is activated appears to differ somewhat from the mechanisms that activate mammalian caspases, which involve either release of Cytochrome c from mitochondria and assembly of an oligomerized Apaf-1/caspase-9 apoptosome (caspase-9 activation), the formation of caspase-8 trimers induced by activation of death receptors (caspases-8 activation), or direct proteolytic activation of downstream executor caspases (such as caspase-3 and caspase-6) by upstream initiator caspases (such as caspase-8 and caspase-9; Budihardjo et al., 1999; Jiang and Wang, 2004; Liu et al., 1996). The *C. elegans* genome has three additional caspase-like genes but it is currently unclear if any of these genes is involved in programmed cell death (Shaham, 1998).

In addition to *egl-1*, *ced-3*, *ced-4* and *ced-9*, several other genes have been implicated in the activation of the apoptotic program during *C. elegans* development, including the *dad-1* gene (*dad*, defender against apoptotic death; Sugimoto et al., 1995), which encodes a protein similar to the mammalian DAD1 protein (Nakashima et al., 1993), and the *icd-1* gene (*icd-1*, inhibitor of cell death; Bloss et al., 2003; Sugimoto et al., 1995), which encodes a protein similar to the beta-subunit of the nascent polypeptide-associated complex (betaNAC). How *dad-1* and *icd-1* might interact with the core killing machinery is currently unclear.
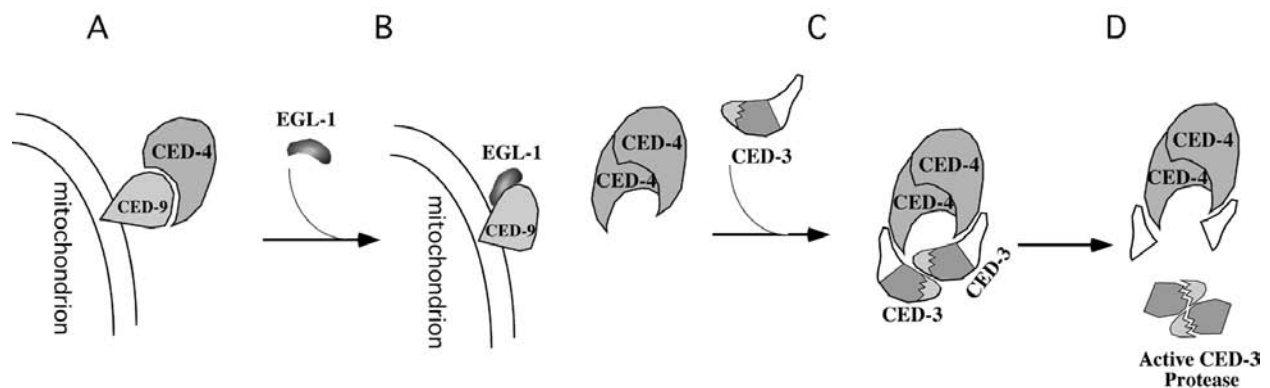
Figure 3. Biochemical model for the activation of programmed cell death. (A) In living cells, CED-4 is tethered to the surface of mitochondria through binding to CED-9. (B) In cells that are doomed to die, the death initiator EGL-1 binds to CED-9, causes a major CED-9 conformational change, and triggers the disassociation of CED-4 from CED-9. (C) Released CED-4 proteins translocate to perinuclear membranes and undergo oligomerization, which brings two CED-3 proenzymes to close proximity. (D) CED-3 proenzymes undergo autoproteolytic activation.

3. Specification phase

Out of the 1090 cells generated during the development of the soma of a *C. elegans* hermaphrodite, 131 undergo programmed cell death (113 of these cells die during embryonic and 18 during post-embryonic development; Sulston and Horvitz, 1977; Sulston et al., 1983). If prevented from undergoing programmed cell

death, the majority of these cells adopt a neuronal fate, indicating that they are of neuronal origin (Ellis and Horvitz, 1986; Ellis and Horvitz, 1991; White et al., 1991). At least some of these "undead" neurons appear to be fully functional (Avery and Horvitz, 1987).

How does a cell know whether to live or die? In higher organisms, cells are generally instructed to die by neighboring cells or by extracellular cues. In contrast, most of the 131 cell deaths observed during *C. elegans* development appear to occur in a cell-autonomous manner; i.e. cells appear to "know" at the time of their birth whether their "fate" is to live or die (Sulston and White, 1980; Evidence for cell non-autonomous induction of cell death exists however in the germ line of *C. elegans* hermaphrodites; (see Germline survival and cell death). The observations that cell fate altering mutations, such as loss-of-function mutations of *unc-86* (*unc*, uncoordinated) or *pag-3* (*pag*, pattern of reporter gene expression abnormal), can affect the essentially invariant pattern of programmed cell death suggest that programmed cell death can be regarded as a cell fate (Cameron et al., 2002; Chalfie et al., 1981; Finney et al., 1988; Sulston and Horvitz, 1981). The current model for cell death specification is that in the 959 cells destined to survive, EGL-1 activity is low or absent and that in the 131 cells destined to die, EGL-1 activity is high. High EGL-1 activity inhibits CED-9 activity, resulting in the activation of CED-4 and CED-3 and the commitment of a cell to the cell death fate (Horvitz, 2003).

EGL-1 activity appears to be regulated at the level of transcription. The *egl-1* gene is expressed predominantly in cells destined to die (Conradt and Horvitz, 1999; Thellmann et al., 2003). Furthermore, mutations in *cis*-acting regions of the *egl-1* locus cause changes in the essentially invariant pattern of developmental cell death (Conradt and Horvitz, 1999). Depending on the particular cells, different transcriptional regulators appear to control *egl-1* expression. For instance, the Zn finger DNA-binding protein and transcriptional repressor TRA-1 (TRA, transformer; Hodgkin, 1987; Zarkower and Hodgkin, 1992) represses *egl-1* expression specifically in the two hermaphrodite-specific neurons (HSNs; Conradt and Horvitz, 1999; Figure 2). Furthermore, the transcriptional activator HLH-2/HLH-3 (HLH, helix-loop-helix transcription factor family), a heterodimer composed of the basic helix-loop-helix (bHLH) proteins HLH-2 and HLH-3 (Krause et al., 1997), is at least partially required for the *egl-1*-dependent death of the NSM sister cells (NSM, neuro-secretory motorneuron; Thellmann et al., 2003). Finally, the mis- or overexpression of the Snail-like Zn finger DNA-binding protein and transcriptional repressor CES-1 (CES, cell-death selection abnormal) in the NSM sister cells inappropriately blocks *egl-1* expression thereby preventing the death of these cells (Metzstein and Horvitz, 1999; Thellmann et al., 2003). *ces-1* expression in the NSM sister cells might normally be repressed by the basic leucine zipper (bZIP) DNA-binding protein CES-2 (Metzstein et al., 1996). These observations suggest that the pattern of *egl-1* expression is the result of the interplay of cascades or networks of positive and negative regulators of transcription. HLH-2/HLH-3, CES-1, and TRA-1 proteins can bind to *cis*-acting regions of the *egl-1* promoter *in vitro* that have been shown to be important for controlling the death of the NSM sister cells (HLH-2/HLH-3, CES-1) or the HSNs (TRA-1) *in vivo* and that are conserved in a related *Caenorhabditis* species, *C. briggsae* (Conradt and Horvitz, 1999; Thellmann et al., 2003). Hence, HLH-2/HLH-3, CES-1, and TRA-1 might be direct regulators of *egl-1* transcription.

At least some of the regulators of *egl-1* expression have additional functions during development. For example, TRA-1 is the terminal, global regulator of somatic sexual fate required for female development (Hodgkin, 1987; Zarkower and Hodgkin, 1992) and HLH-2 is essential for viability (Krause et al., 1997). Some of the cascades or networks that control *egl-1* expression during development appear to be conserved: CES-1-like members of the Snail family of DNA-binding proteins, such as Snail and SLUG, can confer resistance to programmed cell death in mammals and in chick (Inoue et al., 2002; Inukai et al., 1999; Perez-Losada et al., 2003; Vega et al., 2004), and the CES-2-like proto-oncogene HLF (HLF, hepatic leukemia factor) has been implicated in the regulation of SLUG in mammals (Inaba et al., 1996; Inukai et al., 1999). However, *egl-1* might not be the only gene that needs to be regulated at the transcriptional level for proper cell death specification. The proteins EOR-1 and EOR-2 (EOR, enhancer of Raf), which have been proposed to act as regulators of transcription (Howard and Sundaram, 2002), are required for the death of the HSNs in males, however, they are not required for the activation of *egl-1* transcription in these cells (Hoeppner et al., 2004).

4. Execution phase

Once the apoptotic program is activated, it initiates the cell disassembly process, which includes nuclear DNA fragmentation, cytoplasm shrinkage, and exposure of "eat-me" signal(s) on the cell surface to induce phagocytosis by neighboring cells (Steller, 1995).

4.1. Nuclear DNA fragmentation

The fragmentation of chromosomal DNA is a hallmark of apoptosis and may facilitate apoptosis by terminating DNA replication and gene transcription (Arends et al., 1990). DNA fragmentation during *C. elegans* apoptosis has been studied with the aid of various DNA-staining techniques, including DAPI or Feulgen staining (Sulston, 1976) or TUNEL staining (Gavrieli et al., 1992; Wu et al., 2000).

So far ten genes have been identified to be involved in nuclear DNA degradation during apoptosis (Parrish et al., 2001; Parrish and Xue, 2003; Sulston, 1976; Wang et al., 2002; Wu et al., 2000). These include *nuc-1* (*nuc*, nuclease defective), *cps-6* (*cps*, CED-3 protease suppressors), *wah-1* (*wah*, worm AIF homologue), *crn-1* to *crn-6* (*crn*, cell death related nucleases), and *cyp-13* (*cyp*, cyclophilins). Loss or reduction of activity in any of these genes results in the accumulation of TUNEL-positive cells in *C. elegans* embryos, suggesting that these genes are important for resolving TUNEL-reactive DNA breaks generated during apoptosis (Parrish et al., 2001; Parrish and Xue, 2003; Sulston, 1976; Wang et al., 2002; Wu et al., 2000). In addition, reduction of activity in most of these genes (with the exception of *nuc-1* and *crn-6*) causes delayed appearance of embryonic cell corpses during development and reduced cell deaths in sensitized genetic backgrounds, suggesting that nuclear DNA degradation is important for normal progression of the apoptotic process and can even promote cell killing. Genetic and phenotypic analyses indicate that these genes act in multiple pathways and at different stages to promote DNA degradation and apoptosis, with *cps-6*, *wah-1*, *crn-1*, *crn-4*, *crn-5* and *cpy-13* acting in one pathway and *crn-2* and *crn-3* in the other (Parrish and Xue, 2003; Wang et al., 2002). Defects in both DNA degradation pathways not only cause a more severe defect in nuclear DNA degradation but also a synthetic defect in cell corpse engulfment, suggesting that the DNA degradation process may affect cell corpse removal (Parrish and Xue, 2003). In addition to its cell death function, *nuc-1* is involved in the degradation of DNA derived from ingested bacteria in the intestinal lumens (Sulston, 1976; Wu et al., 2000).

Both *cps-6* and *wah-1* encode mitochondrial proteins, which are similar to human mitochondrial endonuclease G (EndoG) and apoptosis-inducing factor (AIF), respectively (Parrish et al., 2001; Wang et al., 2002). Ectopic *egl-1* expression induces WAH-1 translocation from mitochondria to nuclei in a CED-3 dependent manner, suggesting that the role of mitochondria in regulating apoptosis is conserved. The WAH-1 protein can physically associate with CPS-6 and enhance the endonuclease activity of CPS-6 (Wang et al., 2002). Furthermore, CPS-6, CRN-1, CRN-4, CRN-5 and CYP-13, which are either endonucleases or exonucleases, appear to interact and cooperate with one another, possibly in a large DNA degradation complex named degradeosome (Parrish and Xue, 2003), to promote stepwise DNA fragmentation, starting from generating DNA nicks, gaps, to double-stranded DNA breaks (Parrish et al., 2003). Both *nuc-1* and *crn-6* encode type II acidic DNases and do not seem to affect either the activation or progression of cell death or the engulfment of cell corpses (Hedgecock et al., 1983; Parrish et al., 2001; Parrish and Xue, 2003; Wu et al., 2000). These two nucleases may act at later stages of apoptotic DNA degradation, possibly in the lysosomal compartments of the engulfing cell to promote degradation of engulfed apoptotic cells.

4.2. Engulfment of cell corpses

When a cell undergoes apoptosis, "eat-me" signals are rapidly exposed on the surface of the dying cell, which are recognized by engulfing cells (Fadok et al., 2001). The engulfment signal is transduced to the cellular machinery in the phagocyte to trigger the phagocytic process (Hedgecock et al., 1983; Sulston and Horvitz, 1977). Genetic analyses have identified eight genes, *ced-1*, *ced-2*, *ced-5*, *ced-6*, *ced-7*, *ced-10*, *ced-12*, and *psr-1* (*psr*, phosphatidylserine receptor homologue), which appear to function in two pathways to promote the cell-corpse engulfment process, with *ced-1*, *ced-6* and *ced-7* acting in one pathway and *ced-2*, *ced-5*, *ced-10*, *ced-12* and *psr-1* in the other pathway (Chung et al., 2000; Ellis et al., 1991; Gumienny et al., 2001; Wang et al., 2003; Wu et al., 2001; Zhou et al., 2001). Most of these genes act in engulfing cells to promote corpse removal, with the exception of *ced-7* whose activity is required in both the dying cell and the engulfing cells (Reddien and Horvitz, 2004; Wu and Horvitz, 1998a).

ced-1, *ced-6* and *ced-7* appear to encode components of a signaling pathway involved in cell-corpse recognition. CED-1 is similar to the human scavenger receptor SREC and may function as a corpse-recognizing phagocytic receptor since CED-1 protein is found to cluster around cell corpses (Zhou et al., 2001). CED-7 is similar to ABC (ATP-binding cassette) transporters and may play a role in promoting or mediating cell-corpse recognition by CED-1 as CED-1 receptors fail to cluster around dying cells in mutants defective in the *ced-7* gene (Wu and Horvitz, 1998a; Zhou et al., 2001). The CED-6 protein contains a PTB (phosphotyrosine-binding) domain (Liu and Hengartner, 1999), which directly binds to the intracellular domain of CED-1 (Su et al., 2002). CED-6 may act as a signaling adaptor downstream of CED-1 and CED-7 (Figure 4).
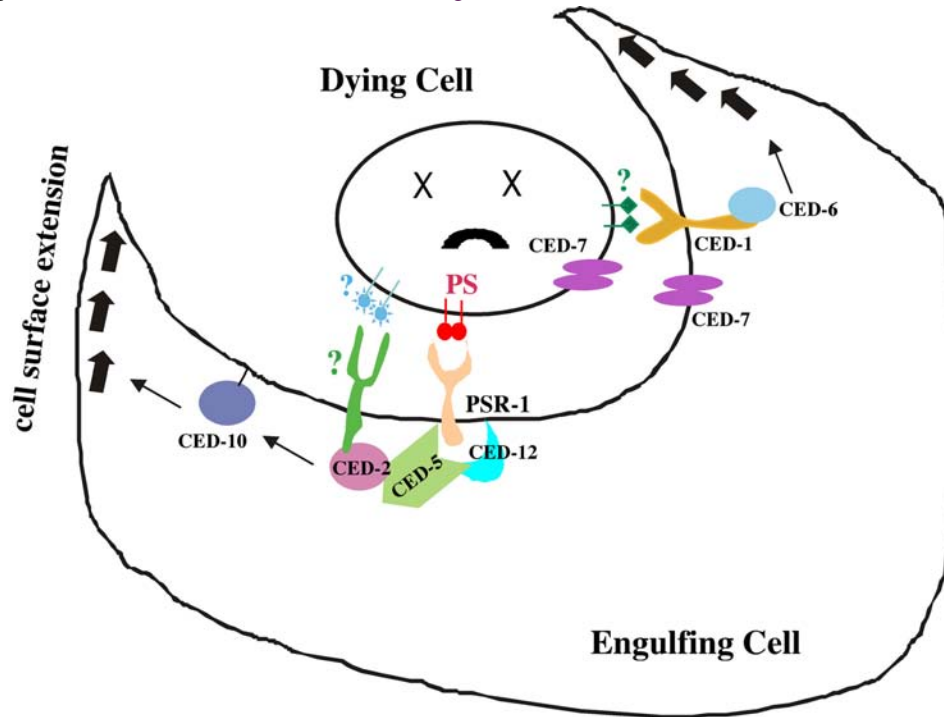
Figure 4. Molecular model for the cell corpse engulfment process. The engulfment process is mediated by two partially redundant pathways. In the CED-1/CED-6/CED-7 pathway, CED-1 and CED-7 act on the surface of the engulfing cell to mediate recognition of an unknown engulfment signal(s) on the surface of the dying cell (green diamonds) and to transduce the signal through CED-6 to activate the phagocytic machinery of the engulfing cell. CED-7 also acts in dying cells. In the CED-2, CED-5, CED-10 and CED-12 pathway, PSR-1 may act in the engulfing cell to mediate the recognition of PS (red circles) externalized by the dying cell and to transduce the signal through the CED-2/CED-5/CED-12 ternary complex to activate CED-10. There are likely other engulfment receptors that act in the CED-2, CED-5, CED-10 and CED-12 pathway.

In addition to mediating corpse engulfment, *ced-2*, *ced-5*, *ced-10*, and *ced-12* control the migration of the gonadal distal tip cells (DTC) and the CAN cells (Gumienny et al., 2001; Reddien and Horvitz, 2000; Wu and Horvitz, 1998a; Wu et al., 2001; Zhou et al., 2001), and the development of D-type motorneurons and amphid sensory neurons (Lundquist et al., 2001; Wu et al., 2002). *ced-2*, *ced-5*, *ced-10*, and *ced-12* encode conserved components of the Rac GTPase signaling pathway involved in regulating actin cytoskeleton rearrangement essential for cell migration and corpse engulfment. CED-2 is a CrkII-like adaptor with one SH2 and two SH3 domains (Reddien and Horvitz, 2000). CED-5 is similar to human DOCK180, which physically interacts with human CrkII (Wu and Horvitz, 1998a). CED-10 is a *C. elegans* homologue of mammalian Rac GTPase (Reddien and Horvitz, 2000), which controls cytoskeletal dynamics and cell shape changes. CED-12 contains a potential PH (pleckstrin-homology) domain and an SH3-binding motif (Gumienny et al., 2001; Wu et al., 2001; Zhou et al., 2001). Genetic analyses indicate that *ced-2*, *ced-5* and *ced-12* appear to function at the same step but upstream of *ced-10* in the engulfment process. Biochemical analyses suggest that CED-2, CED-5 and CED-12 form a ternary complex to activate CED-10 GTPase activity (Brugnera et al., 2002; Chung et al., 2000; Ellis et al., 1991; Gumienny et al., 2001; Lu et al., 2004; Wu et al., 2001; Zhou et al., 2001). The phosphatidylserine receptor-like protein PSR-1 specifically recognizes apoptotic cells with externalized phosphatidylserine (PS), an engulfment-inducing signal (Fadok et al., 2001), and may trigger the formation of the CED-2/CED-5/CED-12 ternary complex and the subsequent activation of CED-10 GTPase by binding to CED-5 and CED-12. Since the *psr-1* mutant displays a significantly weaker engulfment defect than any of the *ced-2*, *ced-5*, *ced-10* mutants, other "corpse-recognizing"

receptor(s) must also act in this pathway (Wang et al., 2003; Figure 4). In addition to CED-10, two other Rac-like GTPases, MIG-2 and RAC-2, also contribute to cell corpse phagocytosis (Lundquist et al., 2001).

Phagocytosis not only removes cell corpses generated by programmed cell death, but also may actively promote apoptosis (Hoeppner et al., 2001; Reddien et al., 2001). Engulfment genes appear to act in engulfing cells to promote apoptosis (Reddien et al., 2001). Furthermore, these two engulfment pathways are also important for the removal of necrotic cell corpses, suggesting that similar mechanisms are used to recognize and remove apoptotic and necrotic corpses (Chung et al., 2000). Several other genes have also been implicated in cell death execution, including the *ced-8* gene which appears to control the timing of cell death and encodes a protein similar to human XK, a putative membrane transport protein (Stanfield and Horvitz, 2000).

5. Conclusions

The genetic and molecular characterization of genes, which, when mutated or inactivated by RNAi, affect developmental cell death in *C. elegans*, has revealed some of the molecular mechanisms involved in the specification, killing or execution phase of programmed cell death. However, much remains to be learnt about programmed cell death. For example, while we know that the transcriptional activation of the *egl-1* gene specifies whether a cell will live or die, very little is known about what regulates *egl-1* expression. Furthermore, while we know that the CED-3 caspase is essential for cell killing and required for DNA fragmentation and engulfment, it is currently not known how CED-3 is activated and what CED-3 substrates are. Finally, while more is known about the molecular components that act in engulfing cells to mediate cell corpse engulfment, little is known about what acts in the dying cell to trigger the phagocytic event and how engulfing cells promote killing in a cell non-autonomous manner. By answering these and other remaining questions, studies of developmental cell death in *C. elegans* will continue to contribute in a major way to our current knowledge of programmed cell death.

6. Acknowledgements

7. References

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